Optimising Technical Protocols to Detect AID Expression in Archived FFPE Samples of Patients with Follicular Lymphoma

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The B-cell mutator, activation induced cytidine deaminase (AID), induces genomic alterations required for immunoglobulin somatic hypermutation and class switch recombination in normal B cells. The mutagenic effects of AID may also contribute to genomic instability in B-cell malignancies.

Measuring AID expression is an attractive approach to study its roles in B-cell malignancies. However, this is hampered in studies on lymphomas, by technical difficulties in obtaining good quantity and quality of RNA and protein in formalin fixed paraffin embedded (FFPE) tissue samples. The aim of our study was to optimize test conditions for the detection of AID mRNA and protein levels in FFPE lymph nodes from 87 patients with FL.

By comparing different RNA extraction kits, we found that the Qiagen RNeasy FFPE kit produced the highest yield of RNA using the smallest amount of FFPE tissue. Although partially degraded, RNAs prepared from 60 of the 87 FFPE samples that had RNA integrity number (RIN) ≥2.1 (2.1-5.1), and were successfully used to quantify AID expression in RT-qPCR with primers targeting a 90-bp sequence.

We used immunohistochemistry (IHC) and a standard scoring system to quantify AID protein expression in FFPE tissue sections from all of the 87 patients. Staining was scored by two independent observers. A significantly positive correlation between the mRNA and protein expression was observed, despite not perfect (r=0.36, p=0.005; Pearson correlation).

To investigate AID subcellular localization, we found that the Hoechst stain with immunofluorescence (IF) produced the best images with confocal microscopy compared to other nuclear stains. We used Image J software, and improved the program’s ability to make a clear nuclear-cytoplasmic distinction and automatically measure subcellular AID in a large number of cells. Using the optimised IHC in a study of 20 patient samples, we found a significantly higher proportion of nuclear AID in patients with high total AID protein (r=0.54, p=0.013; Pearson correlation).

In summary, we have successfully optimised methods for quantification of AID mRNA, as well as total and nuclear proportion of AID protein in archived FFPE samples from patients with FL. These protocols may be also applicable to measure expression of other genes FFPE biopsy materials from other diseases.